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Effects of *Hypericum perforatum* on the healing of xenografts: a histomorphometric study in rabbits

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Abstract

The aim of this study was to investigate effects of the *Hypericum perforatum* (St John's Wort) on bone healing in rabbit calvarium. Ten male New Zealand rabbits each had three bicortical defects made in the calvarial bones, which were filled with xenograft, xenograft + *H perforatum* oil extract, and autogenous graft. Four weeks postoperatively all rabbits were killed and the bony defects examined histomorphometrically. Tissue compartments including new bone ($p < 0.001$), marrow space ($p < 0.001$), and residual bone grafts ($p = 0.014$) differed significantly among groups. The volume of residual graft was significantly decreased in the xenograft/*H perforatum* group compared with those with xenografts alone ($p = 0.0147$). The differences in microarchitectural variables of de novo bone formation were also significant (trabecular thickness ($p < 0.001$), trabecular width ($p < 0.001$), trabecular separation ($p = 0.001$). There were no significant differences in node:terminus ratio between the xenograft/*H perforatum* group and the other two groups. However, the difference in node:terminus ratio between the autogenous graft and xenograft group was significant ($p = 0.001$) Oil extracts of *H perforatum* improved bony healing in defects filled with bovine-derived xenografts.

Keywords: *Hypericum Perforatum*; Xenogreft; Bone Healing

Introduction

Several substitutes for bone grafts have been used to improve both the quantity and quality of bone in the dental implant area.[1] Although autogenous bone grafts are still considered the gold standard, limits to the quantity of grafts that can be harvested, and the need for a secondary surgical site, are disadvantages.[2] The search for alternative biomaterials therefore still continues.

Xenografts, particularly those derived from cattle, have been used in many animal and clinical studies. These demineralised bovine grafts have a structure similar to human bone and have been used for many oral interventions such as augmentation of the alveolar crest, raising the sinus floor, and repair of periodontal defects.[3] The results are acceptable but generally not as good as those of autografts. However, they are better than those of most synthetic biomaterials.[4]

Hypericum perforatum (St John's Wort), the best known of the Hypericaceae family, has been used since ancient times, and contains hypericin, hyperforin, and flavonoids.[5] Olive oil extract of this herb has long been used both orally and topically as a homemade remedy to treat burns, cuts, gastrointestinal ulcers, haemorrhoids, depression, and diabetes

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throughout Europe and Asia.[6] Both in vivo and in vitro studies showed that *H perforatum* shortened the duration of inflammation, stopped the migration of fibroblasts, and increased collagen deposition during wound healing.[7] As far as we know this is the first study to investigate the effects of *H perforatum* on the healing of xenografts.

The purpose of this experimental study therefore was to investigate the efficacy of *H perforatum* on the promotion of bone healing in rabbit calvarial defects treated with bovine bone-derived xenografts.

Material and Methods

Ten skeletally mature (9-12 months) male New-Zealand rabbits (3 (0.5) kg body weight) were used in the study. All the animals were given a standard diet.

H perforatum flowers collected during June and July were dried and ground to a powder. *H perforatum* 50 g was placed in a glass bottle containing olive oil 500 ml. The bottle was kept in the sun for 12 hours a day for four weeks during the summer,[7] during which time the red dye of the plant diffused into the olive oil. High-performance liquid chromatography (HPLC) and gas chromatograph-mass spectrometer (GC-MS)(Shimadzu QP2010 Ultra, Kyoto, Japan) were used to analyse the components of the *H perforatum* oil extract, and the results were compatible with those reported previously.[8] Naftodiantron (0.06-0.4%), phloroglucinols (0,2-4%), flavonoids (2-4%), phenylpropanes (<1%), proanthocyanidins (2-4%) and biflavons are the main components of this plant and essential oils were listed as cyclopentadecanone, 2-hydroxy- (66.75%), palmitic acid (17.61%), cyclopropane octanoic acid (2.53%), and heneicosane (2.27%).

Surgical technique

Animals were anaesthetised with an intramuscular injection of ketamine 35 mg/kg (Ketalar®, Pfizer, Turkey) and xylazine 3 mg/kg (Rompun®, Bayer, Turkey). With the animals prone, the frontal and parietal bones were prepared and draped under aseptic conditions. A supraperiosteal injection of articaine 2 ml (Ultracain-DS®, Hoechst Marion Roussel, Turkey) was given to provide local anaesthesia and haemostasis.

The bone was exposed through a linear incision from the nasal bone to the midsagittal crest. Three craniotomy defects were made with a trephine 8 mm in diameter and 2 mm deep. Bicortical defects were completed with round burs to avoid perforation of the dura. The first defect was filled with xenograft (İntegross bone Plus, Turkey), which was bone substitute consisting of deproteinised bovine bone, with particles 0.5-1 mm in size. The second defect was filled with xenograft that had been soaked in the *H perforatum* olive oil extract for five minutes, and the third defect was filled with autograft that was collected during preparation

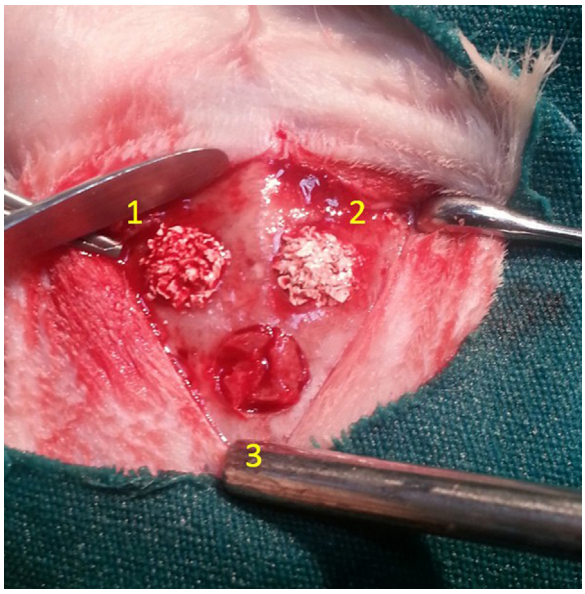

Fig. 1. Intraoperative view of the calvarial defects filled with xenograft + *Hypericum perforatum* extract (1), xenograft (2), and autograft (3).

the defects and crumbled (Fig. 1). After the grafts had been inserted, the defects were covered with resorbable collagen membrane (Collagen AT, Italy) and flaps were closed with 3/0 polyglactin 910 (Vicryl®, Ethicon, USA). Tramadol 1 mg/kg (Contramal®, Abdiibrahim, Turkey) and cefazolin 25 mg/kg (Cefamezin®, Eczacıbasi, Turkey) were each given intramuscularly twice a day for four days. The animals were killed four weeks postoperatively with an intravenous injection of potassium chloride 25 mg/kg (7.5% potassium cloride) under general anaesthesia.

Histomorphometric analysis

The soft tissues overlying the operated site were dissected and the calvarial defects were sectioned carefully with a surrounded 1 mm of healthy bone. The specimens were fixed with 10% buffered formalin, dehydrated with increased concentrations of ethanol (70%-99%) for 10 days, and embedded in methyl methacrylate (Technovit 7200VLC; Haerus Kulzer GmbH, Wehrheiam/Ts, Germany). Transverse sections 40 μm thick were prepared using an electric diamond saw and grinding system (Exakt, Norderstedt, Germany) and stained with toluidine blue. The specimens were then washed with distilled water, dried, and a cover slip applied. Digital images of the slides were obtained with a camera (Olympus® DP 70, Tokyo, Japan) adapted to a microscope x 40 magnification. The images were transferred to a PC and analysed with histomorphometry software (WinTASversion0.1, University of Leeds, UK).